PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

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PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)* see form PCT/ISA/210 (second sheet)	
Applicant's or agent's file reference see form PCT/ISA/220	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB2007/000488	12.02.2007	10.02.2006

International Patent Classification (IPC) or both national classification and IPC
INV. C12N15/85

Applicant
OXITEC LIMITED

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:	Date of completion of this opinion	Authorized Officer
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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/GB2007/000488

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

      ☒ a sequence listing

      ☐ table(s) related to the sequence listing

   b. format of material:

      ☒ on paper

      ☒ in electronic form

   c. time of filing/furnishing:

      ☒ contained in the international application as filed.

      ☒ filed together with the international application in electronic form.

      ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. II Priority

1. ☒ The validity of the priority claim has not been considered because the International Searching Authority does not have in its possession a copy of the earlier application whose priority has been claimed or, where required, a translation of that earlier application. This opinion has nevertheless been established on the assumption that the relevant date (Rules 43*bis*.1 and 64.1) is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-18, 23, 25-36
	No:	Claims	19-22, 24, 37-44
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-44
Industrial applicability (IA)	Yes:	Claims	1-44
	No:	Claims	

2. Citations and explanations

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

1. Additional remarks to item V (reasoned statement under Rule 66.2(a) (ii) with regard to novelty, inventive step or industrial applicability)

1.1 Present application

The present application is directed to a gene expression system comprising splice control sequences. The splice control sequence allows an additional level of control of gene expression by providing a mechanism for alternative splicing in a claimed sex, stage, germline or tissue specific manner. The gist of this application is the sex-controlled expression of lethal genes for the control of an organism population. In other words an heterologous lethal gene is expressed in one sex and not the other by sex specific alternative splicing of the lethal gene. Examples of splice control sequences are sequences derived from tra or dsx intron retaining the alternative splicing function.

1.2 Prior art documents

The present communication refers to the documents cited in the International Search Report (ISR). Said documents are numbered as in the ISR, i.e. D1 corresponds to the first document cited in the ISR. The numbering will be adhered to in the rest of the procedure.

These prior art documents disclose, among other, the following data:

(i) **D1** corresponds to a prior Applicant's application directed to the development of expression systems for insect pest control. These polynucleotide expression systems are characterized by comprising a gene to be expressed and its promoter, where a product of the gene to be expressed serves as a positive transcriptional control factor for the promoter and the product or its expression is controllable. Exemplified are systems comprising tTA gene product combined with tetO operator whose expression is regulated by tetracycline, or a tTA variant denominated tTAV constitutively highly expressed (p.18). Interestingly, example 12 provides pLA1188 (SEQ 22, Fig.17) where Cctra intron within tTAV ORF allows reconstitution of intact tTAV by alternative splicing of intron only in medfly female larvae. According to paragraph 3 of page 42, female larvae yielded PCR products corresponding to the expected sizes that would result from splicing in the pattern of the endogenous Cctra gene. The authors conclude that the Cctra intron can splice correctly in a heterologous context and provides a suitable method for introducing sex-specificity into a positive feedback construct. Thus combination of non sex-specific expression of a transcriptional activator with sex-specific

expression through splicing, of a functional RNA under the transcriptional control of the transcriptional activator allows sex-specific expression of a target gene (Figure 18 and p.13, last paragraph - p.14, paragraph 3).

(ii) **D2** is directed to the identification of the cis-element which is required for the sex-specific splicing of dsx in tissues of Bombyx mori (Bm). It reports that a Bm mini gene consisting of exon 1 and 5 and shortened introns 2 and 4 contains the information necessary for the correct regulation of alternative splicing.

(iii) **D3**studies the molecular mechanisms of sex differentiation in the mosquito Anopheles gambiae with the goal of identifying genes for inducing specific male sterility or for sex-controlled expression of lethal genes. Dsx gene is shown to have a role in determining the sexual fate in A. gambiae. D2 also reports the presence of regulatory elements in the 3' UTR of the female-specific exon (Agdsx RE elements), which display high homology with the Dmdsx splice enhancer dsxRE elements and the Dmfru RE elements (Table 2). These Agdsx RE elements are located much further downstream from the 3' splice acceptor site that in Drosophila, a situation similar to Dmfru RE elements. The authors conclude that the identification of female and male specific transcripts of Agdsx represents an important step towards the understanding of the sex differentiation process in A. gambiae and will facilitate the development of genetic tools to manipulate sex ratios in mosquitoes, for example by inducing the sex-specific splicing of a dominant lethal gene.

(iv) **D4** discloses the identification of an Actin-4 promoter as a female specific promoter in Aedes aegyptii. Said promoter is considered useful for the preparation of transgenic strains of mosquitoes carrying dominant conditional-lethal genes.

(v) **D5** reports the development of plasmids carrying tetracycline repressible transactivator as a transactivator and lethal gene for the control of insect pests population (Sterile Insect Technique), in particular medfly (Med. fruitfly). The results shown that highly efficient, repressible, dominant lethality can be achieved in the medfly using these compact expression systems which give performance characteristics appropriate for incorporation into SIT based control programs. Said system is expected to work across a wide phylogenetic range (easier transfer to other species) because of the absence of

tephritid DNA.

(vi) **D6** corresponds to the late publication of main examples of the current application.

1.3 Statement with regard to novelty and inventive step (Articles 33 (2) and (3) PCT)

1.31 Novelty

The subject-matter of claims **1-18, 23, and 25-36** does not meet the requirements of Articles 33 (2) and (3) PCT, because said claims lack novelty in view of D1 and/or their lack of clarity.

The plasmid pLA1188 (Figure 17, SEQ ID N°22) disclosed in example 12 of D1, corresponds to an expression system for tTAV ORF which comprises an Cctra intron. Since D1 reports that said intron allows reconstitution of intact tTAV by alternative splicing of intron in medfly female larvae only (sex-specific manner) (see § 1.2 i), said plasmid anticipates novelty of **claims 1-6, 8-18, 23 and 25-36**. With regard to claim 13, homologues of a specific promoter is considered to embrace any promoter (Article 6 PCT).

1.32 Inventiveness

The subject-matter of **claims 1-44** does not meet the requirements of Article 33 (3) PCT, because said claims do not involve an inventive step in view of the teachings of D1 combined with D2 and/or D3 and/or D4.

D1 is considered to be the closest prior art, because it already teaches a polynucleotide expression system for the control of insect pests population.

The polynucleotide expression systems of present claims 19-22, 24 and 27 differ from the system disclosed in example 12 of D1 by the presence of a different splice control sequence or a different effector gene (RNAi). Thus the problem to be solved by the present application is to provide an alternative expression system for insect pest control.

However the IPEA considers that it would be obvious for the skilled person trying to solve the above problem to look for alternatives of the Cctra intron used in D1. By doing so he would inevitably start from insect genes, whose expression is known to be sex

specific, such as the dsx genes from Drosophila or Aedes gambiae whose RE elements have already been studied (see D2 and D3, § 1.2 ii and iii respectively) or Actin-4 gene known for comprising a female specific promoter (see D4, § 1.2 iv). Since the "splice control sequence" referred to in the current claims is only characterized by the result to be achieved (see § 2.1), no inventive step can be acknowledged for the intention of identifying further "splice control sequences" in insect genes known for sex-specific expression. Last but not least it is pointed out that the plasmid pLA3077 comprising a Cctra intron-tTAV construct referred to in present claim 24 (SEQ ID N°50) is 98.8 % identical to the whole nucleotide sequence of plasmid pLA1188 disclosed in D1 (which corresponds to SEQ ID N°49 of the present application). In the absence of any unexpected function or technical effect of said pLA3077 versus known pLA1188, the subject-matter of claim 24 consists merely in choosing from a number of equally likely alternatives among the routine trials and the obvious design procedures. Thus, **claims 19-22 and 24** are considered as obvious alternatives for the person skilled in the art trying to solve the above technical problem, and, as such, do not involve an inventive step.

The same objection is raised against inventiveness of **claims 37 and 38-44** because the purpose of D1 is to use its expression system for the control of insect population (see § 1.2 i). It also already teaches the use of RNAi as an effector gene in said expression systems (D1: claim 22). No inventive step can be acknowledged for the claims 1-44 on file.

2. Certain observations on the international application (Articles 5 and 6 PCT)

2.1 Lack of clarity

It is clear from the specification that the "splice control sequence" is the key component of the expression system presently claimed. However said essential feature is only characterized by being "capable of mediating" alternative splicing which should be sex, stage, germline and/or tissue specific in some conditions ("in cooperation with a spliceosome"). Thus the reference to "splice control sequence" corresponds to a definition by the result to be achieved, what is not allowable in the sense of Article 6 PCT. Since the claims on file state the desired result without indicating, in terms of technical features, how this problem is solved, they are considered to lack an inventive step in the sense of **Article 33 (3) PCT**: the solution to a technical problem can not be

the problem itself (see also § 1.32).

The indication of the source of a sequence ("derived from ...") does not limit the scope of the claims 15-22. Actually the source of a sequence is interpreted as a "product by process" feature which is not clear in the sense of Article 6 PCT, because the same sequence may be obtained from different species or different strains of the same species as confirmed by the consensus sequences already proposed for dsx gene RE elements (see, for example, D3, § 1.2 iii).

Thus the claims on file require amendments to limit their scope to polynucleotide expression system characterized by essential technical features which permit to obtain the desired effect, or, differently expressed, which allow the skilled person to put the claimed matter into practise without undue experimentation. The same remark holds true for the possible difference between the claimed system and the pLA1188 plasmid disclosed in D1. The Applicant states in the specification that said prior art plasmid is not capable of allowing the expression of an intact functional tPAV protein. In such case the essential technical features of the claimed expression system leading to this difference in the expression result should be included in the claims.

2.2 Lack of technical support

The above objection for lack of clear technical features leads to an objection for lack of technical support over the whole of the broad field claimed, pursuant to Articles 6 and 5 PCT (PCT Guidelines, Part II, Chapters 5.43-5.45). It is further pointed out that present claims embrace the expression of the heterologous polynucleotide in any organism, i.e. in other words humans, bacteria, plants etc ..., whereas the "splice control sequences" referred to in the application are all derived from a few insect genes whose expression is sex specific. Which spliceosome should be present in the "organism" to ensure the alternative splicing mediated by Cctra intron ?